Tissue Inhibitor of Metalloproteinase-2 Gene Delivery Ameliorates Postinfarction Cardiac Remodeling

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Abstract

Hypothesis: Adenoviral-mediated (AdV-T2) overexpression of TIMP-2 would blunt ventricular remodeling and improve survival in a murine model of chronic ischemic injury. *Methods:* Male mice ($n = 124$) aged 10–14 weeks underwent either (1) left coronary artery ligation to induce myocardial infarction (MI group, $n = 36$), (2) myocardial injection of 6×10^{10} viral particles of AdV-T2 immediately post-MI (MI + T2 group, $n = 30$), (3) myocardial injection of 6×10^{10} viral particles of a control adenovirus (MI + Ct, $n = 38$), or 4) received no intervention (controls, $n = 20$). On post-MI day 7, surviving mice ($n = 79$) underwent echocardiographic, immunohistochemical, and biochemical analysis. *Results:* In infarcted animals, the MI + T2 group demonstrated improved survival ($p < 0.02$), better preservation of developed pressure and ventricular diameter ($p < 0.04$), and the lowest expression and activity of MMP-2 and MMP-9 ($p < 0.04$) compared with MI and MI + Ct groups. All infarcted hearts displayed significantly increased inflammatory cell infiltration ($p < 0.04$ vs. control, MI, or MI + T2), with infiltration highest in the MI + Ct group and lowest in the MI + T2 group ($p < 0.04$). *Conclusions:* Adenoviral mediated myocardial delivery of the TIMP-2 gene improves post-MI survival and limits adverse remodeling in a murine model of MI. Clin Trans Sci 2011; Volume 4: 24–31

Keywords: remodeling, gene therapy, metalloproteinases

Introduction

Cardiac remodeling (left ventricular dilation and fibrosis) consists of short- and long-term responses to myocardial injury that underlie the mortality and morbidity associated with heart failure. Therapeutic interventions that preserve cardiac function and survival in the postmyocardial infarction (MI) setting also limit cardiac remodeling.[1] Important mediators of the proteolytic mechanisms associated with post-MI processes of cardiac rupture and LV dilation are the matrix metalloproteinases, particularly the so-called "gelatinases" MMP-2 and MMP-9. MMP-2 and -9 (as well as other MMPs) show complex temporal and spatial heterogeneity of waxing and waning expression/activity in the heart after ischemic injury.[2] In rodent models of ischemic injury arising from coronary artery ligation (CAL), cardiac MMP-2 and MMP-9 both show rapidly enhanced expression/activation (within hours to 3 days post-MI), that remains elevated throughout the early wound healing (0–7 days post-MI) and early remodeling (7–21 days post-MI) intervals.[3–5] The naturally occurring inhibitors of MMPs (TIMPs1–4) also show similarly complex responses to cardiac ischemic injury with divergent reports regarding the congruent changes in TIMP RNA and protein.[4,6,7] Nonetheless there is a consensus that the increase in MMP-expression/activation exceeds that of TIMP protein expression, leading to increased MMP activity, enhanced proteolytic effects, and remodeling of the ischemic myocardium.[8] These concepts arise from the observed relative and temporal changes of MMPs and TIMPs in ischemic hearts,[4,6,7] the beneficial effects of genetically deleting MMP-2 or MMP-9 expression,[3,9] the detrimental effects of deleting TIMP-1, TIMP-2, and TIMP-3,[10–12] and the beneficial effects of reduced cardiac remodeling with utilization of therapies that inhibit MMP activity.[13–16] Thus ischemic injury and remodeling (both short- and long-term) is associated with and likely mediated by changes in the MMP/TIMP ratio, among other mechanisms.

Substantial efforts have sought to devise and utilize synthetic MMP inhibitors so as to limit the adverse effects on cardiac remodeling and other diseases processes mediated by MMPs. While several agents have shown beneficial effects in limiting postinfarction cardiac remodeling in animal,[13–16] a concern for off-target and adverse effects (joint remodeling, myalgias, arthralgias),[17,18] and limited efficacy in clinical trials[19] have blunted enthusiasm for these compounds.[20] Interestingly, individual TIMPs have differential effects on cell proliferation, death, inflammation, and wound-healing[21,22] that may occur independently of their interactions with MMPs, and a variety of non-MMP binding partners of TIMPs have been identified and implicated in these processes.[23–25] Therefore, altered expression of TIMPs may provide beneficial therapeutic effects not observed with synthetic MMP-inhibitors, and several studies have utilized a gene therapy approach to modify expression of specific TIMPs in various models of cardiovascular disease and remodeling. For example, in a murine model of atherosclerosis, systemic adenoviral-mediated overexpression of either TIMP1 or TIMP2 blunted the development of unstable plaques, although greater benefit was observed with TIMP-2 overexpression.[26] Furthermore, transient adenoviral-mediated overexpression of TIMP-1 limited cardiac remodeling preserved diastolic and systolic function, and reduced collagenase activity and fibrosis in rodent model of MI.[3,27] As TIMP-1 and TIMP-2 show differential biologic effects, and differential cardiac expression in response to cardiac injury and heart failure,[6,7,28] we sought to test the hypothesis that adenoviral-mediated cardiac overexpression of TIMP-2 would be beneficial in a murine model of cardiac ischemic injury.

Methods

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the *International Journal of Cardiology*.[29] Animal research was performed with

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the approval of the University of Pittsburgh Institutional Animal Care and Use Committee.

Generation of adenovirus encoding TIMP-2

The study utilized a replication-defective adenoviral vector expressing murine TIMP-2 under the control of the cytomegalovirus promoter (AdVTIMP2). Briefly, a shuttle vector containing full-length murine TIMP-2 cDNA alone was co-transformed with adenoviral backbone plasmid pAdEasy-1 for homologous recombination in *E. coli* BJ5183. Positive recombinants were linearized and transfected into 293 cells for virus packaging and propagation. Adenoviruses were purified by CsCl banding, dialyzed, viral particle concentration determined by spectophotometry, and stored at –80°C until use. An "empty" vector virus (AdY5, University of Pittsburgh Vector Core Facility) that encoded no recombinant proteins served as a control.

Myocardial infarction and gene delivery

A total of 124 C57/BL6J male mice (12 ± 2 weeks old; weight 25 ± 7.24 grams) obtained from Jackson Laboratories (Bar Harbor, ME, USA) were utilized in the study.

MI was induced in three groups of mice; MI group, MI with control adenovirus (MI + Ct group), myocardial infarction with adenovirus encoding TIMP-2 (MI + T2 group). MI was induced as previously described.[4] The fourth group (control, CTL) mice received no surgical intervention.

Adenoviral gene delivery for TIMP-2 or control virus was performed immediately after induction of myocardial infarction. The infarct and peri-infarct zones were identified by myocardial blanching, and the position of the coronary ligature. Based on preliminary experiments, a multisite injection strategy was chosen to ensure a uniform distribution of virus. Nine injections (a total of 6×10^{10} viral particles in 27 μL) were delivered directly into the myocardium in the infarct and peri-infarct zones. After surgery mice received analgesia with buprenorphine (0.05 mg/kg) intraperitoneally, given every 12 hours for 48 hours. Animals that died within 24 hours of surgery (~3–5%) were excluded from the study as surgical error could not be excluded as the cause of these deaths.

Functional assessments

All mice were examined at 7 days postinfarction. Transthoracic echocardiography was performed usingVevo 770 Echocardiogram machine, as described,[4] to assess LV end diastolic diameter and fractional shortening. Next, a 1.4 Fr conductance catheter (Millar Instruments, Houston, TX, USA) was used as previously described[4] to measure left ventricular ejection fraction, diastolic volume, and d*P*/d*T*.

Tissue Harvest and Processing

Mice utilized for histological analysis were euthanized while under avertin anesthesia by lethal intravenous injection of potassium chloride (2 mmoL/kg) to arrest the heart in diastole. Hearts were washed in PBS, and the atria and right ventricle removed before weighing. For biochemical analysis, the left ventricle was dissected under an operating microscope and the infarct and peri-infarct zones employed for biochemical analysis as described.[4]

For protein extractions, tissue fragments were homogenized in a solution containing 0.05M Tris-Cl (pH 7.5), 0.2% Brij-35, 0.075M NaCl, and 1 μL/mL proteinase inhibitor (cat# P8340, Sigma, St. Louis, MO, USA) centrifuged and the supernatant analyzed for total protein concentration by the method of Bradford.

Immunohistochemistry and *in situ* zymography

Excised ventricles (n = 4 in each group) were rinsed in cold phosphate-buffered saline, cut in half and frozen in supercooled 2-methyl butane (liquid nitrogen bath). Frozen sections (6 micron thick) were cut by cryostat and sections interacted with rabbit anti-mouse collagen I (1:1000 dilution, catalog #AB765, Chemicon, Billerica, MA, USA), rat anti-mouse CD45 (1:50 dilution, cat# 01111D BD Biosciences, Franklin Lakes, NJ, USA), DRAQ5 to identify nuclei (1:1000 dilution, cat BOS-889–001, Axxora, San Diego, CA, USA), or phalloidin to identify filamentous actin (1:250 dilution, cat# A22287 Molecular Probes, Carlsbad, CA, USA). Fluorescent secondary antibodies included goat anti-rabbit immunoglobulin G conjugated with Alexa488 (1:500 dilution, cat# A11034, Molecular Probes) and goat anti-rat conjugated with Cy3 (1:1000 dilution, cat# A11034, Jackson Immuno Research, West Grove, PA, USA). *In situ* zymography was performed as previously described.[30] Slides were kept at 4°C until visualization and image capture on a FV 1000 laser scanning Confocal microscope (Olympus, Center Valley, PA, USA) with 40× objective (1.3 NA). Images from different wavelengths were collected with a cooled CCD camera at 24-bit gray depth and assembled (Adobe Photoshop, Adobe, San Jose, CA, USA). Relative gelatinase activity, collagen I percent fractional area, and inflammatory cell counts (CD45+ cells) were performed by determining the area of each microscopic field stained with respective color dye as a percentage of the area stained with phalloidin (to index tissue area), as previously described.[4] The analysis of an entire cross-sectional plane of the LV was integrated so as to minimize quantitative errors arising from sampling biases (e.g., heterogeneity of inflammation or fibrosis between the peri-infarct and remote zones).

Western blotting

Ten micrograms of total extracted protein from each sample was separated on 12% SDS-PAGE and electroblotted onto nitrocellulose membrane (Micron Separations, Westborough, MA, USA). MMP-2 and 9 controls were included and electrophoresed in parallel with the myocardial samples. Immunoreaction with primary antibodies [anti-MMP-9, 1:500 (cat# AB16306 Abcam, Cambridge, MA, USA), anti-MMP-2 1:500 (Chemicon cat# MAB3308), and anti-GAPDH 1:2000(RDI cat# RDI-TRK5G4–6C5)] was followed by enhanced chemifluorescence (ECF) detection (cat# RPN5780 Amersham, Piscataway, NJ, USA), and scanned using a Storm PhosphorImager (Molecular Devices; GE Healthcare Life Sciences). Relative intensity of the specific protein expression, normalized to GAPDH expression was determined using ImageQuant 5.1 (Molecular Dynamics).

MMP and TIMP Assays

Matrix metalloproteinase activity (MMP) was measured using 100 micrograms of protein per sample in chromogenic MMP-2 and -9 activity assays (cat# RPN2617 and RPN2634, respectively, GE Lifesciences, Piscataway, NJ, USA), according to the manufacturers protocol. These assays use a specific substrate for MMP-2 and -9, respectively. The samples were activated using *p*-aminophenylmercuricacetate (APMA) according to the manufacturer's suggestion. Results obtained via spectrophotometry were compared against serial dilutions of known concentrations of the respective standards. Results were expressed in nanograms of MMP/100 micrograms protein. TIMP-1 and TIMP-2 protein levels were measured using ELISA

kits (cat# MTM100 R&D Systems, Minneapolis, MN, USA and GE Lifesciences, cat# RPN2618), according to the manufacturer's instructions using 100 μg of protein per sample. Results obtained via spectrophotometry were compared against serial dilutions of known concentrations of the respective standards.

Data Analysis

Data are reported as mean ± standard deviation (SD). Western blot results were presented as percent change compared with controls, the means of which were arbitrarily set as 100%. Using automated quantification software (Metamorph Molecular Devices), the summed area of the protein/activity of interest as detected by a particular fluorescent color was divided by the total area surveyed (calculated by magnification power, size of field, number of fields, and corrected for the area within each field occupied by tissue), and expressed as a percentage (fractional area). Results of functional and biochemical tests were compared between groups by a nonparametric one-way analysis of variance using the Kruskal Wallis test. Upon detection of overall significance, limited hypothesis driven *post hoc* analyses were performed using Mann–Whitney U test. Kaplan–Meier survival curves were generated and differences in survival assessed using the log-rank statistic. (SPSS, Chicago IL, USA). A *p*-value of less than 0.05 was considered significant.

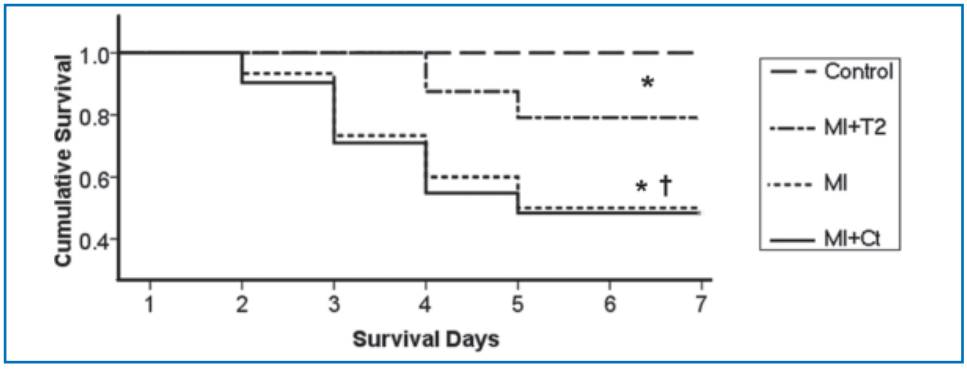

Figure 1. Survival (Kaplan–Meier) after myocardial infarction. Controls ($n = 20$), MI ($n = 36$), MI + Ct ($n = 38$) and MI + T2 ($n = 30$). Post-MI survival was significantly improved in the MI + T2 group vs. MI or MI + Ct. *$p < 0.04$ vs. control; †$p < 0.04$ vs. MI + T2.

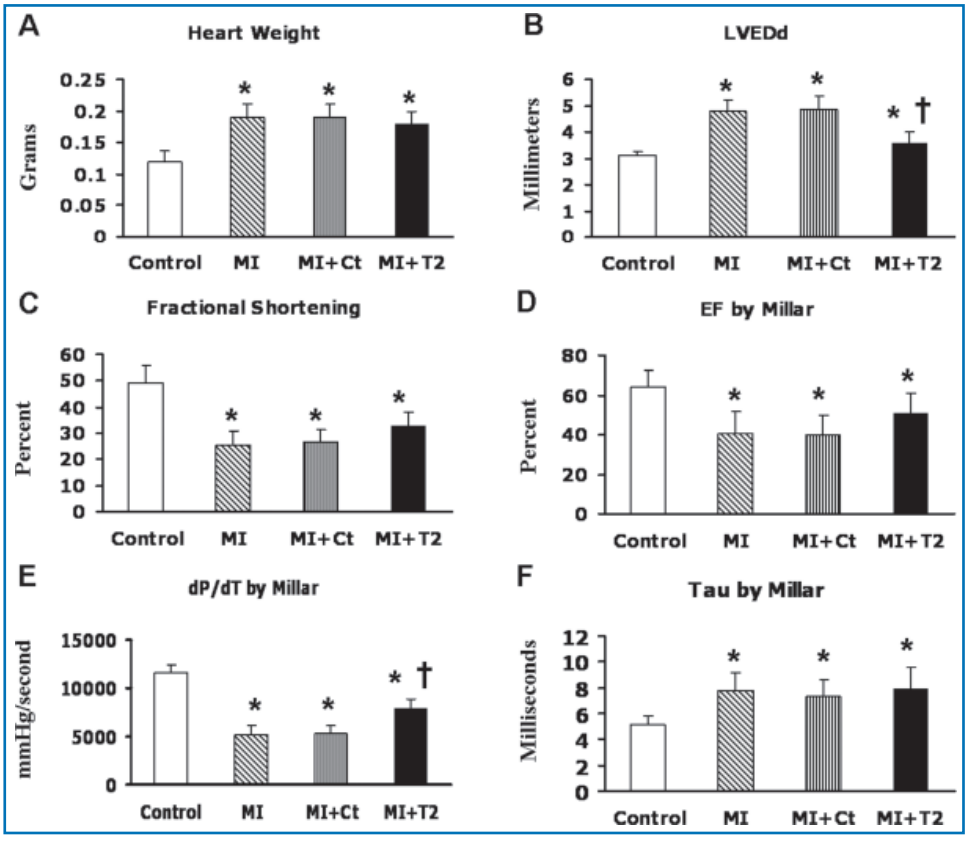

Figure 2. Morphometry and functional analysis. (A). Biventricular weight was increased similarly in all infarcted groups compared with control. (B) Left ventricular diameter as measured by echocardiography was increased in all infarcted groups compared with control, but was best preserved in the MI + T2 group (control: $n = 20$; MI: $n = 18$; MI + Ct: $n = 18$; MI + T2: $n = 20$). (C). Fractional shortening as measured by echocardiography was decreased in all infarcted groups compared with control. (D). Ejection fraction measured by conductance catheter was significantly worse in all infarcted groups compared with control, and showed a trend ($p < 0.09$) toward improvement in MI + T2 relative to MI and MI + Ct groups ($n = 5$ in each group). (E). Developed pressure as measured by conductance catheter was decreased in all MI groups, but was best preserved in the MI + T2 group. (F). Tau, a measure of diastolic performance was similarly impaired in all infarcted groups compared with control ($n = 5$ in each group). *$p < 0.04$ vs. control; †$p < 0.04$ vs. MI + T2.

Results

Survival is improved with TIMP-2 gene delivery

Overall surgical mortality (death within 24 hours of surgery) was <5% (six animals). These animals were excluded from the study, as surgical error could not be ruled out as their cause of death. Seventy-nine mice survived till the end of the study period. Survival was significantly better in the MI + T2 group (76.7%) compared with the MI (50%) or the MI + Ct group (47%) ($p < 0.04$; *Figure 1*); there were no deaths in the control group. There was no significant difference between the MI and MI + Ct group. Most (>80%) of deaths that occurred more than 24 hours after surgery appeared to arise from LV rupture as evidence by a tear in peri-infarct myocardium and/or copious amounts of clotted blood in the chest cavity; the incidence of rupture as a cause of death was not different between the infarcted groups.

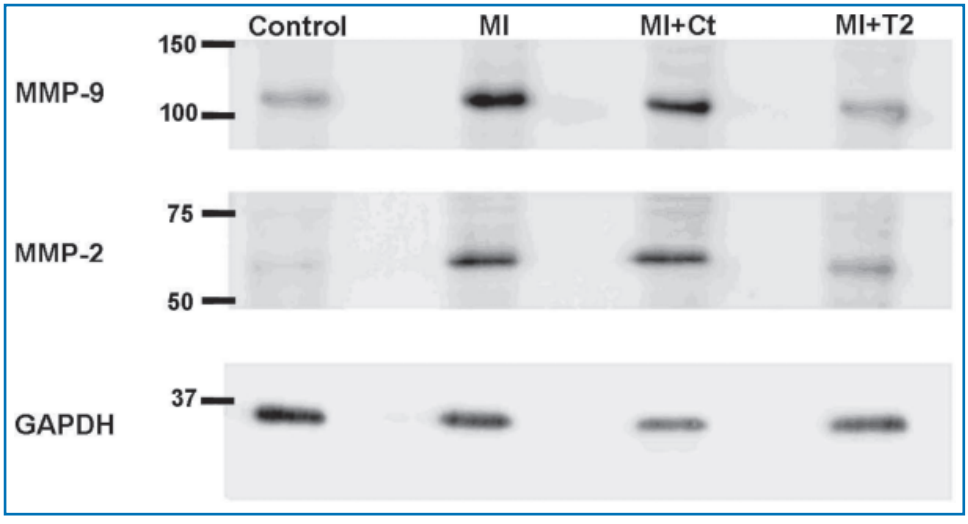

Figure 3. Representative western blots of MMP-2, -9 and GAPDH, with molecular weight markers.

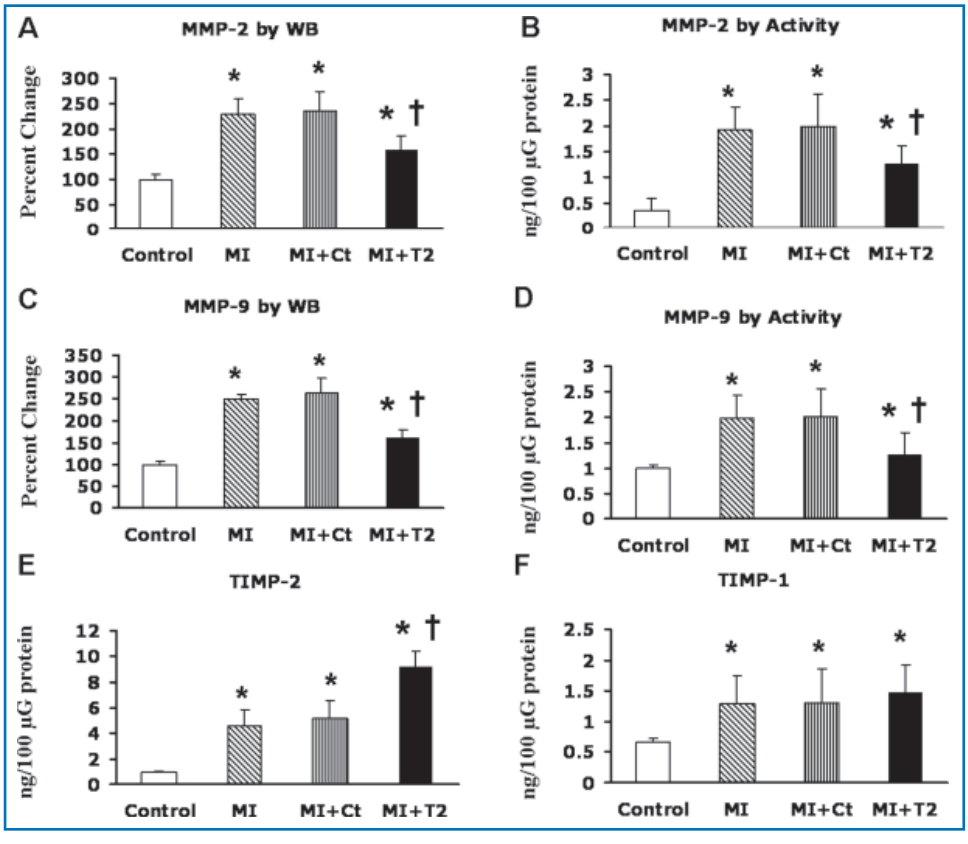

Figure 4. MMP and TIMP levels in the myocardium. By Western blotting (A and C), and activity assay (B and D), MMP-2 and -9, respectively, were increased in all infarcted groups compared with control. However, levels and activity were least altered in the MI + T2 group. TIMP-2 was highest in the MI + T2 group (E), while TIMP-1 was not differentially altered in any of the infarcted groups compared with controls. $N = {}^{*}p < 0.04$ vs. control; ${}^{\dagger}p < 0.04$ vs. MI + T2.

TIMP-2 gene delivery does not reduce postinfarction cardiac hypertrophy

Left ventricular hypertrophy as assessed by biventricular weights was increased in all infarcted groups compared with control (*Figure 2A*). However, there was no significant difference seen between any of the infarcted groups. Similarly, biventricular weight/body weight ratios were increased in all infarcted groups compared with control, but not differentially altered between the various infarcted groups (data not shown).

TIMP-2 gene delivery ameliorates postinfarction cardiac dilatation

At the time of echocardiographic and conductance catheter measurements, measured heart rate was similar between all groups. LV diastolic diameter measured by TTE was increased in all MI groups. However, the MI + T2 group had decreased dilatation compared with MI and MI + Ct. (*Figure 2B*). Fractional shortening by TTE was significantly lower in all MI groups, and showed a trend towards impairment in MI and MI + Ct animals relative to MI + T2 group ($p < 0.08$) (*Figure 2C*). Notably, infarct size as measured by TTE was not different between the MI groups (data not shown).

Cardiac performance (assessed by ejection fraction (EF) and developed pressure) was measured by conductance catheter. EF was significantly worse in all infarcted groups compared with control, and showed a trend ($p < 0.09$) towards improvement in the MI + T2 group relative to the MI and MI + Ct groups (*Figure 2D*). Developed positive pressure ($+dP/dT$) was significantly worse in all infarcted groups compared with control. However, the MI + T2 group showed a significantly ($p < 0.04$) better preservation of $+dP/dT$ compared with MI and MI + Ct groups (*Figure 2E*). $+dP/dT$ corrected for systolic blood pressure was similarly better preserved in the MI + T2 compared with MI and MI + Ct groups ($p < 0.04$; data not shown). To confirm that this protection was not offset by impaired diastolic performance, tau (τ) was also determined. This was not significantly different between any of the infarcted groups, but the performance of the infarcted groups was impaired compared with control (*Figure 2F*).

TIMP-2 gene delivery reduces postinfarction MMP activity

Representative western blots are shown in *Figure 3*. MMP-2 protein levels as assessed by western blotting were increased in

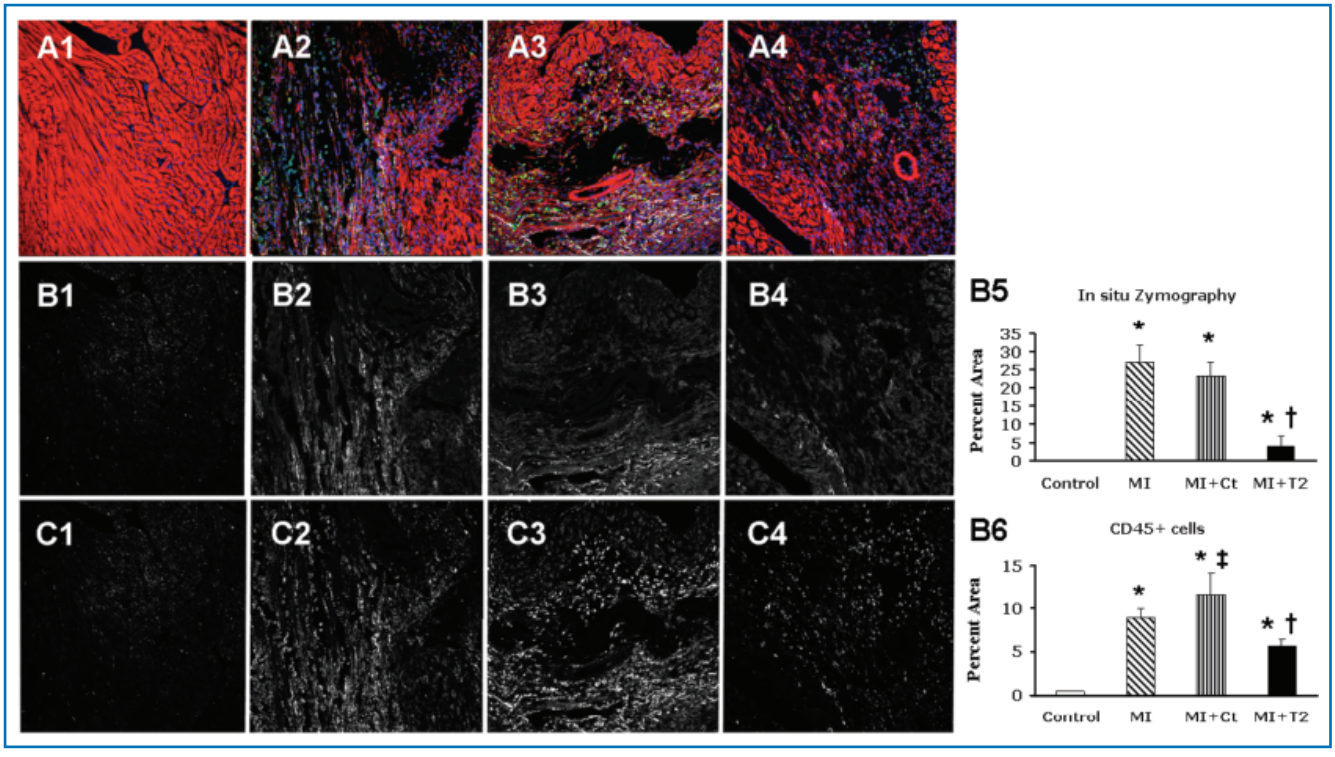

Figure 5. Representative images of *in situ* zymography and immunohistochemistry. Four-color images of control (A1), MI (A2), MI + Ct (A3) and MI + T2 (A4) in the peri-infarct zone, with phalloidin (red) staining for myocardium, DRAQ5 (blue) for nuclei, CD45 (green) for inflammatory cells, and gelatinolytic (MMP-2 and -9) activity (white). Panels B1–4 demonstrate corresponding single channel images for gelatinolytic activity, and panel B5 shows quantitative data. Panels C1–4 demonstrate corresponding single channel images for inflammatory cells (CD45+), and panel C5 shows quantification of CD45 + "area." *$p < 0.001$ vs. control; †$p < 0.01$ vs. MI + T2; ‡$p < 0.05$, vs. MI and MI + T2 groups.

all the infarcted groups compared with controls. However, levels were significantly lower in the MI + T2 group compared with MI or MI + Ct group (*Figure 4A*). Similarly, MMP-9 protein levels were increased in all the infarcted groups, but were lower in the MI + T2 group (*Figure 4C*). By activity assays, total (i.e., APMA-activated) MMP-2 and -9 activity was increased in all infarcted groups compared with control. However, activity for both MMPs were lowest in the MI + T2 groups (*Figure 4B* and *D*).

Cardiac TIMP-2 levels, as expected, were highest in the MI + T2 group, but were also increased in the Mi and MI + Ct groups compared with control (*Figure 4E*). TIMP-1 on the other hand was increased in all the infarcted groups compared with controls, but was not significantly different between the infarcted groups (*Figure 4F*).

In order to estimate the actual activity of gelatinases within the heart, *in situ* zymography was performed (*Figure5A* and *B*). All infarcted groups (*Figure 5B2–4*) displayed an increase in gelatinolytic activity compared with control (*Figure 5B1*). However, this activity appeared lower in the MI + T2 groups (*Figure 5B4*) compared with MI and MI + Ct groups (*Figure 5B2* and *3*). Quantitative analysis of the percent fractional area showing gelatinolytic activity was significantly reduced in the MI + T2 group when compared to MI or MI + Ctl mice (*Figure 5B5*).

TIMP-2 gene delivery reduces postinfarction inflammatory cell infiltration, but not collagen deposition

We also examined major components of infarct healing; infiltrating cell density and fibrosis as measured by CD45 positive cell densities (*Figure 5A* and *C*) and collagen I deposition. Infarcted animals were characterized by a marked inflammatory cell (CD45+) infiltration (*Figure 5C2–4* vs. *Figure 5C1*). The MI + T2 group appeared to have a notably lower CD45 + cell count when compared with the MI and MI + Ct groups (*Figure 5C4* vs. *Figure 5C2* and *3*). Quantitative assessment of CD45 "area" showed a significant decrease in the MI + T2 group when compared to MI or MI + Ctl mice, although CD45+ infiltration was still significantly higher than in the control mice (*Figure 5C5*). On the other hand, the MI + Ct showed the highest area occupied by CD45+ cells. Collagen I deposition as assessed by immunohistochemistry was not significantly different between the infarcted groups, although all MI groups had increased collagen deposition compared with control (*Figure 6*).

Discussion

This study sought to test the hypothesis that transient adenoviral-mediated overexpression of TIMP-2 would limit cardiac remodeling and preserve cardiac function in a murine model of myocardial chronic ischemic injury. TIMP-2 differs from TIMP-1 (and other TIMPs) in a number of ways including the effects on cell proliferation and migration, stimulation of collagen and myofibroblast differentiation, and ability to inhibit or activate processing of particular MMPs.[21,22,26,31–33] In addition, TIMP-2 can participate in a trimolecular complex with MT1-MMP and proMMP-2 and allow free MT1-MMP to process pro- to active MMP-2. However, a further increase of TIMP-2 levels can inhibit all MT1-MMP and MMP-2.[34,35] Therefore, while increased TIMP-2 expression may prove more beneficial in atherosclerotic plaque stabilization than overexpression of TIMP-1,[26] and while TIMP-2 deficiency may worsen remodeling,[12] the effect of increased

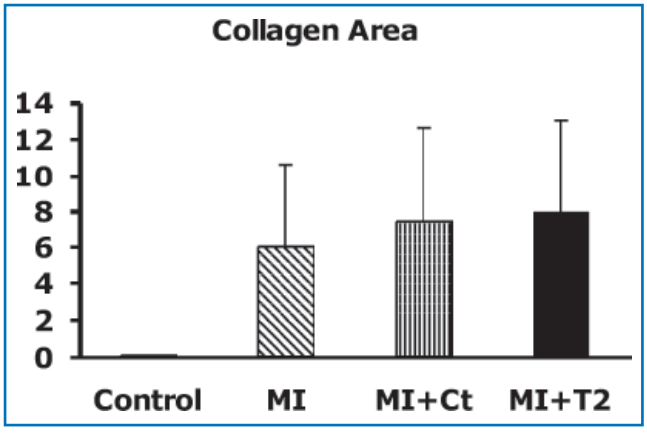

Figure 6. Collagen I content, measured by immunohistochemistry was increased in all infarcted groups compared with control; however, there was no difference between any of the infarcted groups.

TIMP-2 expression in the actively remodeling ischemic heart is uncertain.

The approach utilized direct cardiac injection of adenoviral constructs encoding TIMP-2 so as to achieve a local increased expression of TIMP2. While preliminary studies with adenovirus encoding the intracellular beta-galactosidase reporter demonstrated little spread beyond the site of injection, the release of a soluble protein such as TIMP-2 was likely more widespread. To control for the potential pro-inflammatory effects of adenoviral injections, we included a series of mice that received an "empty" adenovirus (AdY5) vector that did not encode foreign proteins. Injections were made at the time of inducing ischemic injury via CAL, with maximal TIMP-2 expression expected 5–7 days later. Several important observations were made.

First, increased expression of murine TIMP-2 was achieved in CAL mice receiving AdVTIMP-2, which was not observed in CAL mice receiving AdY5 or CAL alone. Second, improved survival was observed 1 week post-CAL, which appears to arise from a reduced frequency of LV rupture in the CAL mice. These results are similar to that observed in mice with systemic overexpression of TIMP-1 achieved through adenoviral delivery.[3] The increased TIMP-2 expression did not modify endogenous TIMP-1 expression, but significantly decreased MMP-2 and MMP-9 expression and activity, and *in situ* gelatinolytic activity, demonstrating the inhibitory effect of TIMP-2 overexpression on these gelatinolytic MMPs. Studies utilizing overexpression of adenovirally encoded TIMP-1 had previously observed a decreased gelatinolytic activity when cardiac homogenates were examined,[27] although no observations were performed on MMP-2, -9 expression or *in situ* gelatinase activity of cardiac tissues. Third, TIMP-2 overexpression lead to a significant decrease in CD45 + infiltrating cells, resembling that observed in CAL-mice treated with systemic overexpression of adenovirally-encoded TIMP-1,[3] an MMP-2-specific inhibitor, or in MMP-2 null.[36] Interestingly, the expected time course of TIMP-2 expression derived from adenoviruses[26,37] and the time course of post-CAL CD45 + cell infiltration, activity of MMP-9, and cellular expression of MMP-9 activity closely match[3–5] suggesting that the decreased level of infiltrating cells gave rise to decreased tissue levels of MMP-9. A decrease in lymphocyte infiltration, as well as a potential for anti-proliferative effects on cells such as fibroblasts which may express MMP-[22,25] could explain the decreased MMP-2 expression that was also observed. Interestingly, we did not observe any changes in total LV collagen I deposition in CAL mice that had received AdVTIMP2, which suggests a dichotomy between the *in vitro* effects of TIMP-2 on cardiac fibroblasts (increased collagen deposition[21]) and hearts subjected to chronic ischemic injury.

Most importantly, mice receiving AdVTIMP2 showed significantly better preservation of cardiac function and a reduction in cardiac dilation (improved developed LV pressure and LVED) at 1 week post-CAL. A trend towards a better preservation of systolic function (EF and FS) was also noted. None of the beneficial effects of improved survival, reduced MMP-2/-9 expression or activity, reduced gelatinase activity, reduced CD45+ cell infiltration, or preservation of cardiac structure or function were observed in CAL mice that received the "empty" adenoviral construct, and injection of AdVTIMP2 did not increase TIMP-1 expression, suggesting that the enhanced expression of TIMP-2 blunted the cardiac structural and functional remodeling after CAL.

It is striking that our observations of direct cardiac overexpression of TIMP-2 resembles results from systemic or cardiac-directed overexpression of TIMP-1 in similar rodent models of chronic ischemic injury[3,27] although neither study examined both survival and cardiac structural/functional effects within the same group of animals. This is the first study to assess whether TIMP-2 overexpression has beneficial or detrimental effects in survival and cardiac function in the same cohort of mice subjected to cardiac ischemic injury. This issue is important as TIMP-1 and TIMP-2 can have differential effects on cell growth and death, differential inhibition or activation of various MMPs, and differential expression in the development of heart failure. We observed that, like TIMP-1, overexpression of TIMP-2 in the post-CAL interval can have beneficial effects on cardiac structure and function, and improve survival.

Unlike transient overexpression of TIMP-1[27] we did not observe substantial alterations in collagen deposition in the infarcted myocardium in response to TIMP-2 overexpression, which may be either beneficial in strengthening the wound or detrimental in altering wound compliance. However, differences in methods of assessing collagen/fibrosis, and the choice of time points for analysis after ischemic injury weakens the ability to make direct comparisons between the effects of TIMP-1 and TIMP-2 overexpression on cardiac fibrosis.

The study does contain several limitations. First, we have not assessed whether greater expression of TIMP-2 would lead to further reductions in cardiac remodeling. Second, we only examined a single time point after infarction, during a phase of active remodeling. Whether beneficial results were sustained in the late periods of postinfarction remodeling is unknown. However, the lack of evidence for alterations in collagen deposition quantity, and evidence for short-term improvements in cardiac volume and developed pressures would be expected to predict better long-term outcomes and reduced remodeling. We have not examined the full gamut of MMP and TIMP expression/activity changes that may occur in response to enhanced cardiac TIMP-2 expression. While increased TIMP-2 expression did not alter TIMP-1 expression and did decrease MMP-2 and MMP-9 expression/activity, altered expression of additional matrix-remodeling proteins is a distinct possibility.

We also do not know all of the mechanism by which TIMP-2 overexpression achieves these effects. In particular, TIMP-2 can serve as an important inhibitor of MMP-2. Clearly inhibition (or absence)

of MMP-2/-9 expression and activity could limit extracellular matrix degradation and cardiac remodeling. However, a reduced generation of matrix degradation products may also lead to reduced macrophage infiltration. MMP-2 null mice subjected to CAL show preserved (relative to WT CAL mice) cardiac structure and function. The absence of MMP-2 activity blocks generation of ECM degradation products which typically induce macrophage infiltration.[36] TIMP-2 (but not TIMP-1) can also inhibit MT1-MMP, which is important not only for pro-MMP-2 processing, but also for ICAM-mediated monocyte transendothelial migration. Macrophages are important sources of cardiac MMP-9 after the induction of cardiac ischemic injury, and blockade of leukocyte infiltration limits cardiac rupture.[3] While MT1-MMP expression was not examined in this study, it reportedly does not change in rodent models consequent to CAL,[6,7] supporting the possibility that an increased ratio of TIMP-2 to MT1-MMP was achieved in this study. Importantly, the sequential appearance of neutrophils and macrophages in the postinfarction interval reflects their different roles in wound healing.[38] Whether polymorphonuclear cells, as a component of early infarct infiltration, or monocytes/macrophages, as a later component of infarct healing, are differentially altered in response to transient TIMP-2 overexpression awaits further examination.

In conclusion, adenoviral mediated overexpression of TIMP-2 is beneficial in improving survival and preserving cardiac function, and in limiting cardiac dilation and infiltration in a murine model of cardiac ischemic injury. Such approaches may provide beneficial therapeutic options to modify cardiac remodeling after ischemic injury. Significant further investigations may be warranted to identify the differential efficacy among the four recognized TIMP forms, complete elucidation of their short and long-term beneficial (or detrimental) effects when overexpressed, and identification of their mechanisms of action, which may include alteration of matrix remodeling and cellular infiltration.

Acknowledgments

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